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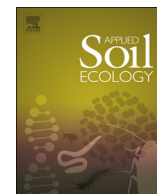
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## Short communication

## Impact of a single freeze-thaw and dry-wet event on soil solutes and microbial metabolites

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## ABSTRACT

Freeze-thaw and dry-wet cycles are common phenomena in temperate regions. Such events may have a significant influence on the functioning of the soil microbial community. Using non-targeted metabolomics, we compared the effects of a single freeze-thaw or dry-wet event on microbial metabolism in an agricultural soil with and without plants. We showed that a dry-wet cycle had a greater impact on solute and metabolite concentrations in the unplanted soil than a freeze-thaw cycle. Drying or freezing caused increases in dissolved organic C, sugars and polyols, suggesting enhanced microbial production to alleviate temperature or moisture stress. Increased nucleobase concentration in the unplanted soil after a dry-wet cycle, and increased amino acids following both stresses, suggested a breakdown of microbial DNA and proteins released from damaged cells. The impacts of stress on metabolites in the planted soil were less than in the unplanted soil. In conclusion, our findings indicate that the soil microbial community responds quickly to stress events by accumulating osmotic solutes (e.g. sugars and polyols) and that a freeze-thaw event causes less disruption than dry-rewetting, and that plants have a key role in the mitigation of the freezing or drying effects on soil microbial communities.

Soils in many agroecosystems frequently experience temperature and moisture extremes (e.g. freeze-thaw and dry-wet cycles; Brabson et al., 2005). These can negatively affect soil biogeochemical cycling, leading to reduced plant productivity and a decline in the delivery of a range of ecosystem services (Sanghera et al., 2011; Fahad et al., 2017). Although plants and soil microbes are sensitive to cold and desiccation stress, there is strong evidence suggesting that many soil microorganisms can adapt to mild freeze-thaw or dry-wet events (Gusta et al., 1997; Craine et al., 2012; Schimel et al., 2007). Typically, this response is underpinned by the synthesis and bioaccumulation of low molecular weight metabolites (e.g. amino acids, sugars and polyols) (Yancey et al., 1982; Cushman, 2001; Yancey, 2001). Studies in pure cultures have shown these mechanisms to be highly effective at reducing cell damage upon exposure to a range of mild abiotic stresses (Schimel et al., 2007). However, the response in actual soil microbial communities remains much less well understood.

In this study, we compared the effects of a single freeze-thaw or dry-wet event on the soil microbial community's metabolite profile. We hypothesized that the microbial response to these two common abiotic stresses would be similar given that both induce osmotic stress and would be characterized by an accumulation of specific solutes. We further hypothesized that the response would be exacerbated in the

presence of plant roots due to an increase in soil microbial biomass and activity in the rhizosphere (Nannipieri et al., 2008), and the release of solutes from damaged root cells.

An agricultural soil (5–10 cm depth, Ah horizon) was collected from a sandy clay loam textured Eutric Cambisol located at the Henfaes Experimental Station, Abergwyngregyn, UK (53°14'22"N, 4°00'60"W). The mean annual air temperature is 10.6 °C (max 28.6 °C, min −7.6 °C) and the mean annual rainfall is 1055 mm y<sup>−1</sup>. A single pre-germinated *Brassica napus* L. seedling (radicle 2 mm long) was placed into individual planted microcosms containing 1 g field-moist Eutric Cambisol (Table S1; Miura et al., 2019) in 5 cm<sup>3</sup> polypropylene tubes (Fig. S1). *Brassica napus* was chosen as it represents a typical winter biennial plant, with high cold tolerance during the vegetative stage (Xin et al., 2019). The plants were grown in a climate-controlled chamber (light intensity of 300 μmol m<sup>−2</sup> s<sup>−1</sup>, 12 h photoperiod, 10 °C) for 2 weeks (shoots 5 cm long). Unplanted microcosms contained only soil. This soil has been shown to have an active microbial community at the low temperatures used here (Farrar et al., 2012). For the freeze-thaw treatment, microcosms were placed at −5 °C for 24 h, and naturally thawed at 10 °C. Using a thermocouple, we estimated that complete thawing occurred within 20 min (Fig. S2). For the dry-wet treatment, air was passed over the samples at a rate of 0.85 m s<sup>−1</sup> (10 °C) until

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they had reached an air-dry state (ca. 3 h when ca. 65% of water was lost) and the plants had begun to wilt within 1 h. After drying (24 h), deionized water was added back to the soil to reach the pre-drying water content (10 °C). Plants survived both the freezing and the drying periods and visually showed no lasting signs of damage. To evaluate changes in metabolite profile, microcosms from each treatment were destructively harvested after 24 h exposure to freezing or drying (i.e. during the stress itself) and the 3 h after thawing or rewetting (i.e. on removal of the stress). In addition, an untreated (control) set of samples was taken immediately before thawing or re-wetting commenced.

In total, there were 9 independent replicates for each treatment with 4 used for soil chemical analysis and the remaining 5 used for metabolomic analysis. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a Multi N/C 2100/2100 analyzer (AnalytikJena AG, Jena, Germany) following extraction of soil (1 g) for 10 min with 5 ml deionized water. A one-way ANOVA with Tukey post-hoc test was used to identify significant differences between soil solution within treatments using a cutoff value of  $P < 0.05$  R Studio 0.99.486 (R Development Core Team, 2004).

Samples for metabolite analysis were immediately frozen in liquid N<sub>2</sub>, freeze-dried and extracted in 3:3:2 (v/v/v) isopropanol/acetonitrile/water according to Fiehn et al. (2008). Non-targeted primary metabolism analysis was performed using an Automated Linear EXchange-Cold Injection System (ALEX-CIS) GC Time of Flight (TOF) MS according to Fiehn et al. (2008). Metabolomics data were pre-processed using ChromaTOF (v2.34; Leco Corp., St Joseph, MI). Subsequent analyses were completed using MetaboAnalyst v4.0 (Chong et al., 2018).

With the exception of unplanted soils subjected to freeze-thaw where TDN decreased, DOC and TDN concentrations in planted and unplanted soils after freeze-thaw or dry-wet events were greater than controls (Fig. 1). The dry-wet cycle increased DOC in unplanted soil more than freeze-thaw, but the relationship was reversed in the planted soil. Metabolite analysis detected 352 individual compounds, of which ca. 44% were positively identified and included a range of sugars, polyols, amino acids and nucleosides (Table S2). Concentrations of sugars and polyols (maltotriitol maltotriose, myo-inositol and

sophorose) and fatty acids (isoheptadecanoic acid, linolenic acid and palmitoleic acid) were increased by drying and re-wetting in the unplanted soil (Fig. 2). Various amino acids in the unplanted soil also increased after the freeze-thaw or dry-wet events. All nucleosides detected in the dried and re-wetted unplanted soil were higher than in controls and other treatments, but were not affected by either stress event in the planted soil. Following drying and re-wetting, a greater number of metabolites were found to increase in the unplanted soil than in the planted soil. However, some sugars and polyols in the planted soil increased in response to drying and re-wetting, including some which were not increased where plants were absent (tagatose, lyxitol, ketohexose, fructose and erythrose). In contrast to the dry-wet event, following freeze-thaw a greater increase in sugars (fructose, erythrose, ketohexose, sophorose, and tagatose) was observed in the planted soil than in the unplanted soil treatments.

The increase in fatty acids in unplanted soil in response to drying stress is consistent with previous stress response studies in microbial cultures and contrasting soil types (Pádrová et al., 2016; Ding et al., 2019). These changes in microbial cell membrane composition have been implicated in promoting greater membrane fluidity, maintaining transport processes, enhancing protection against reactive oxygen species and facilitating energy production (Welte and Gould, 2017; Pádrová et al., 2016; Králová, 2017). The changes in solutes are also consistent with active metabolism even at low temperatures in this soil (Farrar et al., 2012) and plant species (Rapacz and Janowiak, 1998; Xin et al., 2019).

In this study, we found that drying strongly affected cell membrane structure, however, we found no evidence of the same response to freezing stress. We found that free nucleobases increased in concentration after a dry-wet event, suggesting depolymerisation/degradation of RNA and DNA (Rubbi and Milner, 2003) and/or transcriptional inhibition resulting in major changes in nuclear structures (Boulon et al., 2010; Bensaude, 2011). We also found that free amino acids (and soluble N) increased in concentration following both dry-wet and freeze-thaw events, suggesting an increase in proteolysis. Increases in solutes and metabolites following removal of stresses may indicate that some accumulated osmotic compounds were released into the soil

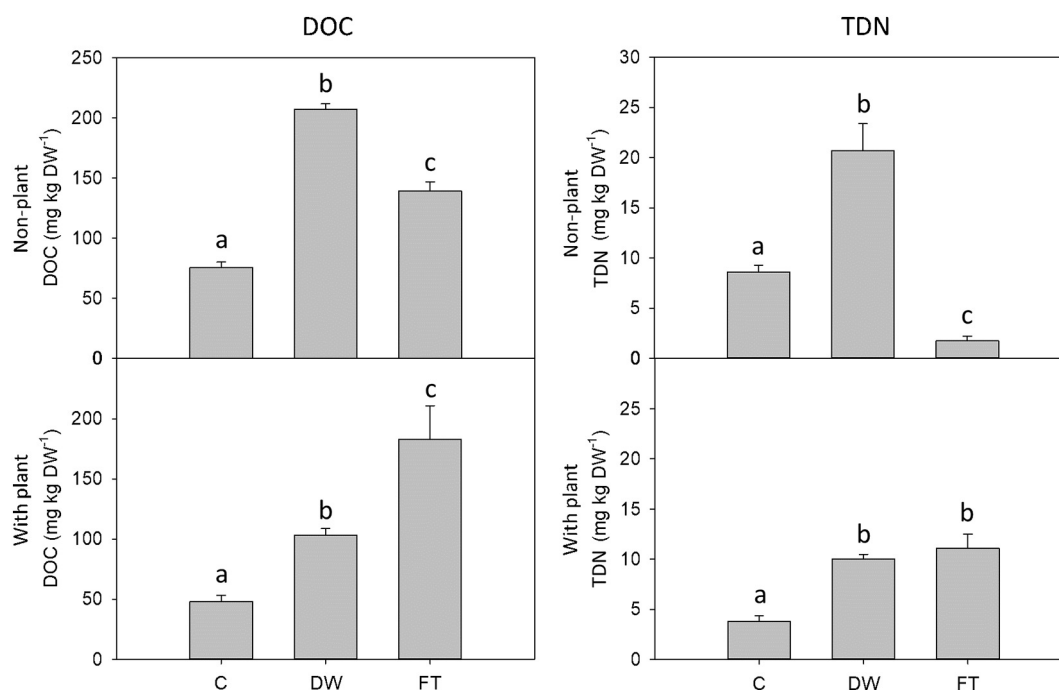
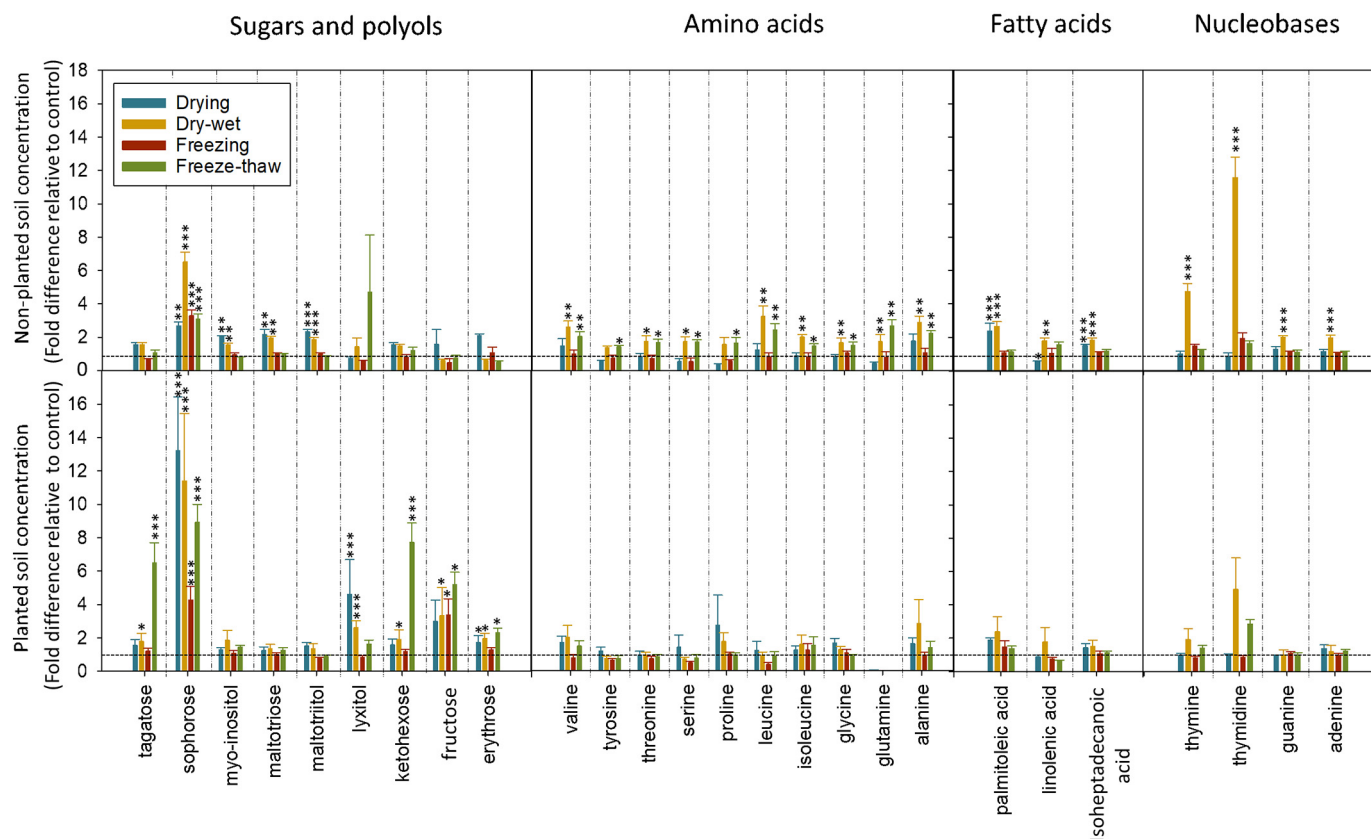


Fig. 1. Dissolved organic C (DOC) and total dissolved N (TDN) concentrations in the planted and non-planted soil (water extract) exposed to either a single freeze-thaw or wet-dry cycle. Values represent means  $\pm$  SEM ( $n = 4$ ). C = Control; DW = Dry-wet; FT = Freeze-thaw. Different letters indicate significant differences between treatments at the  $p \leq 0.05$  level.



**Fig. 2.** Concentration of sugars, polyols, fatty acids, and nucleobases identified in the planted and non-planted soil both during (drying, freezing) and immediately after exposure to a single freeze-thaw or wet-dry cycle relative to concentration in the un-stressed control treatment. The dotted horizontal line represents the metabolite concentration before applying drying or freezing. Stars above the plots denote significant differences from the control where \*, \*\* and \*\*\* denote  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$  respectively. Values represent means  $\pm$  SEM ( $n = 5$ ).

by microbes to prevent cell rupture (Schimel et al., 2007). However, the lack of an effect due to drying or freezing alone, suggests that amino acids were not accumulated as osmolytes. It may also indicate that re-wetting and thawing gave rise to a rise in enzymatic cleavage of proteins and nucleic acids, which cannot occur during the drying event, perhaps due to a lack of liquid water. The smaller effect of freeze-thaw than drying and re-wetting may suggest that soil microbes can remain active at  $-5^\circ\text{C}$  (Clein and Schimel, 1995) and that significant unfrozen liquid water is available in the soil at this temperature (Brooks et al., 1997; Foster, 2015).

Compared with soil without plants, drying and re-wetting of planted soil had a smaller impact on DOC, soluble N and most of the measured metabolites, indicating that soil microbes adapted better to extreme drought when roots were present. Similarly, there was no evidence of increased proteolysis or nucleic acid damage due to stresses where plants were present. The increase in some polyols and sugars only where plants were present suggests a plant response to stress, or microbial accumulation of sugars where C availability is increased due to the presence of living roots. We observed increased DOC and various sugars in planted soil after thawing. We speculate that these sugars are not stress-specific osmolytes present in either plants or soil microbes as we saw little or no change in their concentrations during freezing. It seems more likely that these increases in sugars resulted from damage to plant membranes. However, the lack of increase in other metabolites, especially those indicative of cellular damage (e.g. organic acids, amino acids), may indicate that sugar and polyol provided effective protection to microbes. Thus, it seems likely that, although soil microbes appear to respond rapidly to freezing, and especially to drying, the presence of roots has an important role in the mitigation of the most severe effects on soil microbial communities.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2020.103636>.

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